Optimizing Conditions to Investigate Interactions of an RNA-binding Protein in the Pathogenic Fungus Candida albicans

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Candida albicans is a pathogenic fungus that causes mild infections in healthy people and more serious infections in immunocompromised patients [1]. C. albicans has two cell structures: a budding yeast cell and filamentous cells called hyphae. The yeast form facilitates Candida circulation in the host bloodstream, whereas the hyphal form allows C. albicans to attach to and invade host cells [1]. Thus, the ability to switch from the yeast to the hyphal form is crucial to Candida virulence. For proper hyphal development, certain proteins need to be present at a hyphal tip, and copies of genes (messenger RNAs or mRNAs) for some of those proteins are transported to the hyphal tip to be templates for protein production. The process of mRNA localization in C. albicans could influence the function of hyphal cells.

RNA binding protein complexes transport mRNAs to different locations in a cell, but little is known about these complexes in C. albicans. Absence of the RNA-binding protein Slr1 (SR-like RNA-binding protein 1) slows hyphal formation and decreases Candida virulence. Slr1 localizes in the nucleus whereas a mutant form slr1-mut is found in the cytoplasm and at the hyphal tip. This result suggests that Slr1 shuttles between the nucleus and hyphal tip but returns so quickly that it can only be detected in the nucleus [2]. Slr1 may therefore influence hyphal function by helping transport mRNAs that encode hyphal proteins to the hyphal tip; however, the exact function of Slr1 is not yet understood.

Since Slr1 and slr1-mut localize to different locations in Candida cells, they may interact with different proteins. Thus, one approach to examine Slr1 function is to identify other proteins in Slr1 and slr1-mut complexes and compare these complexes in both yeast and hyphal cells, a project which I started last summer. Slr1 and slr1-mut proteins tagged with green fluorescent protein (GFP) were purified using an immunoprecipitation (IP) technique. With this technique, C. albicans cells were lysed and Slr1-GFP and slr1-mut-GFP complexes were bound to beads with antibodies specific to GFP. Then the beads with the protein complexes were isolated. Individual proteins in the complex were separated by size using gel electrophoresis, and the unknown proteins were analyzed to determine their identities. Last summer I purified Slr1-GFP and slr1-mut-GFP complexes from yeast Candida cells, but with the same IP protocol, I was unable to purify these complexes from hyphal cells. Instead, I detected background molecules that appeared to be found only in hyphal cells, potentially interfering with the purification. Thus, this summer I wanted to optimize purification procedures used to detect Slr1 protein complexes in hyphal cells so that I can continue identifying other proteins in Slr1-GFP and slr1-mut-GFP complexes and compare their interacting proteins in both cell forms of C. albicans.

Last summer, I induced Candida hyphal growth using a growth medium with calf serum, which mimics a human host environment. Thus, the induced hyphal cells may express adherence proteins, which help the fungus attach to the host cells but can also bind to the beads and may block them from binding to the anti-GFP-antibodies. Thus, I tested different hyphal induction methods: a medium with low glucose content (RPMI) and a medium containing N-acetyl glucosamine. Inducing hyphal growth in RPMI, I successfully purified slr1-mut complex from hyphal cells in the absence of the background molecules. I also tested two new types of beads to examine their capacity of purifying slr1-mut complex from hyphal cells. A bead called GFP-Trap appeared to purify more slr1-mut complex than other types, but quantification of this result is required. As now I can purify the slr1-mut complex from hyphal Candida cells, the next step is to analyze the complex and identify unknown proteins. Future identification of these proteins will allow us to learn more about Slr1 function, which hopefully will lead to a better understanding of C. albicans hyphal formation and virulence.

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References